

**Irrigation with Controlled Water Stress and its Effects in ‘Navelate’  
orange plants**

**Irrigação com estresse hídrico controlado e seus efeitos em laranjeiras  
‘Navelate’**

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**ABSTRACT**

Variations in the water regime promote changes in physiology and plant phenology by modifying patterns of growth and development. The aim of this research was to study physiological responses and changes in phenology of ‘Navelate’ orange trees submitted to different intensities of water stress in greenhouse. Stressed conditions were based on 50% and 25% of the field capacity. Gas exchange [photosynthesis (A), transpiration (E), stomatal conductance (gs), internal CO<sub>2</sub> concentration (Ci), water use efficiency (WUE)] were analyzed in addition to growth parameters during 210 days. A completely randomized design was used, the results were submitted to variance analysis and when detected statistical difference, the means were compared by Tukey test at 5% probability. Water stress affected all gas exchange parameters of the exposed orange trees, limiting growth in diameter and height. Submission to amount of water lower than 50% of field capacity undertakes gas exchange, reducing plants growth and development and fruit production.

**Keywords:** Citrus, gas exchange, photosynthesis, water stress, fruit production and phenology.

## RESUMO

Variações no regime hídrico promovem mudanças na fisiologia e na fenologia das plantas, modificando os padrões de crescimento e desenvolvimento. O objetivo desta pesquisa foi estudar respostas fisiológicas e alterações na fenologia de laranjeiras 'Navelate' submetidas a diferentes intensidades de estresse hídrico em casa de vegetação. As condições estressadas foram baseadas em 50% e 25% da capacidade de campo. Trocas gasosas [fotossíntese (A), transpiração (E), condutância estomática (gs), concentração interna de CO<sub>2</sub> (Ci), eficiência no uso da água (WUE)] foram analisadas, além de parâmetros de crescimento durante 210 dias. Utilizou-se delineamento inteiramente casualizado, os resultados foram submetidos à análise de variância e, quando detectada diferença estatística, as médias foram comparadas pelo teste de Tukey, a 5% de probabilidade. O estresse hídrico afetou todos os parâmetros de troca gasosa das laranjeiras expostas, limitando o crescimento em diâmetro e altura. A submissão a uma quantidade de água menor que 50% da capacidade de campo realiza trocas gasosas, reduzindo o crescimento e desenvolvimento das plantas e a produção de frutas.

**Palavras-chave:** Citrinos, trocas gasosas, fotossíntese, estresse hídrico, produção de frutos e fenologia.

## 1 INTRODUCTION

Water stress is one of the most frequent environmental factor which limits orange (*Citrus sinensis*) crop expansion in several places around the world. This water deficit can be resulted by a severe soil water deficit or by an excessive water loss through transpiration in relation to water absorption by root system. In trees, a high evaporative atmosphere demand promotes high transpiration rates, influencing leaf water potential because of low hydraulic conductivity of root system (Jones et al., 1985).

Study of physiological parameters, such as photosynthetic and transpiration rates, and stomatal conductance are very important to evaluate drought tolerance in several plant species by elucidating changes on both production and fruits quality. Citrus under severe water stress close their stomata in order to reduce water loss through transpiration. Those plants can also limit CO<sub>2</sub> diffusion to substomatal cavity, resulting in photosynthetic rate reduction and leaf temperature increase (Ricklefs, 2012).

Phenology studies the occurrence of repetitive biological events and their relationship with changes in the biotic and abiotic environment, contributing to plant regeneration and reproduction understanding (Morellato, 1991). According to Reich (1994), variations in precipitation influence phenology through effects on soil moisture and plant water status.

Southern Brazil, which is one of the orange production areas, has subtropical climate with low temperatures during winter season and both warm and drought during summer season. Under these conditions, it seems to be necessary to study about citrus crop response to water stress, analyze how to choose a better water management during drought season and because of worldwide climate changes. Evaluation of gas exchange and water state of citrus plants can indicate the best conditions to keep both water and carbon balance during drought season in this region.

The aim of the research was to study physiological responses and changes in phenology of 'Navelate' orange trees submitted to hydric stress in greenhouse conditions "with stress" and "without stress

## **2 MATERIALS AND METHODS**

The experiment was conducted in greenhouse of Fruit Crop Sector, Federal University of Pelotas, Brazil (Latitude 31°52'00" S; Longitude 52°21'24" W; altitude 13 m). The climate of this region is Cfa, according to Köppen-Geiger climate classification.

Evaluations were done from August 2014 to March 2015 in 'Navelate' orange trees. Young plants were obtained from commercial nurseries and cultivated in 26 L pots, and received the same irrigation amount during acclimation period of 45 days. After this period, plants received distinct water management: control (substrate humidity corresponding 100% of field capacity), T – 50 (substrate humidity corresponding 50% of field capacity), and T – 25 (substrate humidity corresponding 25% of field capacity).

Gas exchange analyzes, growth measurement, and phenology evaluations were done after acclimation period, monthly during 210 days.

Gas exchange analyzes were done according to following parameters: photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and internal CO<sub>2</sub> concentration (Ci). Evaluations were done by using an infrared gas analyzer model Li-6400 (Portable Photosynthesis System LICOR, Nebraska, USA), with a photosynthetic active radiation intensity of 1200 μmol.m<sup>-2</sup>.s<sup>-1</sup>, measured in previously selected completely expanded leaves. The evaluations were recorded when the coefficient of variation (CV) was less than 0.5% and in temporal stability. Water use efficiency (WUE) and intrinsic water use efficiency (IWUE) were calculated according to follow equations:  $EUA (\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}) = \text{Photosynthesis} / \text{Transpiration}$ , and  $IWUE (\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}) = \text{Photosynthesis} / \text{Stomatal Conductance}$ , respectively.

The plant height (cm) was evaluated, adopting, as criterion, the graft distance until the terminal shoot of the main branch; the plants trunk diameter was determined 5 cm above the graft, using a scale and a digital caliper, respectively.

Experimental design was completely randomized, with three replications in each experimental unit. Values of each parameter was submitted to analysis of variance, compared by Tukey's test at 5% significance and showed as average.

### 3 RESULTS AND DISCUSSION

During this experiment, it was not observed changes on gas exchange and plant phenology until 100 days after started treatments of controlled water stress. After this period, it was observed reduction in this parameter according to the treatments: T-25 < T-50 < Control. Changes were also observed in water use efficiency, leaf temperature and vapor pressure deficit in plants submitted to controlled water deficit in comparison to control plants (Table 1).

One of the first reaction of orange plants grown under severe stress can be the closing of stomatal pores closure, in order to minimize water loss (Larcher, 2000; Taiz and Zeiger, 2013). The tendency of transpiration rate reduction observed in all treatments coincided with significant stomatal conductance reduction throughout the experiment (Table 1). Direct correlation between transpiration and stomatal conductance were expected because of reduction on vapor flux to atmosphere caused by the closing of stomatal pores.

Table 1. Average values of photosynthetic rate (A), stomatal conductance (E), transpiration rate (gs), internal CO<sub>2</sub> concentration (Ci), and intrinsic water use efficiency (IWUE) in 'Navelate' oranges submitted to different levels of irrigation [substrate humidity corresponding 100% (Control), 50% (T-50), and 25% (T-25) of field capacity], evaluated monthly.

Treatments	A ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )	E ( $\text{mmol.m}^{-2}.\text{s}^{-1}$ )	gs ( $\text{mol.m}^{-2}.\text{s}^{-1}$ )	Ci	IWUE ( $\text{mmol.mol}^{-1}$ )
Control	6.05 <sup>a</sup>	3.06 <sup>a</sup>	0.06 <sup>a</sup>	206.88 <sup>c</sup>	1.98 <sup>ns</sup>
T- 50	5.40 <sup>a</sup>	2.64 <sup>b</sup>	0.05 <sup>a</sup>	282.47 <sup>a</sup>	2.01 <sup>ns</sup>
T- 25	4.80 <sup>a</sup>	2.41 <sup>b</sup>	0.04 <sup>a</sup>	231.78 <sup>b</sup>	1.99 <sup>ns</sup>

\*Values followed by same letter did not differ at the 5% probability level by Tukey's test.

Water management affected gas exchange in plants submitted to water stress. It was observed significant differences in photosynthetic rates, stomatal conductance, transpiration rates, and internal CO<sub>2</sub> concentrations (Table 1).

Under water stress conditions it was observed highest values of water use efficiency, probably because of stomatal conductance reduction and consequently decrease the photosynthetic rate when compared to control (Table 1). Ma et al. (2004) concluded that high photosynthetic rate associated with lower stomatal conductance and transpiration rate are typically observed in plants which tolerate lower water availability, but it was not clearly observed in this experiment.

It was occurred reductions on photosynthetic rate (10.74% for T-50, and 20.66% for T-25, both compared to Control), indicating that CO<sub>2</sub> assimilation rate was affected by water stress conditions (Table 1). Internal CO<sub>2</sub> concentrations were higher in plants under water stress, probably because of closing the stomatal pore, resulting in both lower transpiration and respiration rates. The intrinsic water efficiency levels did not differ significantly between treated plants and control, but it was higher under water stress conditions. It could be related to lower levels of stomatal conductance. Larcher (2000) indicated that the best correlation between CO<sub>2</sub> absorption and water consumption occurred when the stomatal pores were partially closed in the beginning of water stress condition. At this time, diffusion processes are promptly reduced, and the intrinsic water efficiency levels reaches the highest values. So, under severe water stress, dehydration of mesophyll cells strongly inhibited plant metabolism and photosynthesis (Taiz and Zeiger, 2013).

Internal CO<sub>2</sub> concentrations, in general, followed gas exchange, showing values lower than the control. The capacity to maintain physiological activity throughout reduction of water availability result in some consequences, such as changes on gas exchange. In fact, it was observed changes on photosynthetic rate and reductions on stomatal conductance in plants submitted to higher deficit of atmospheric vapor pressure. This lower level of stomatal opening, which occurred in plants submitted to water stress, was a consequence of decreased turgor pressure in cells, a higher deficit of atmospheric vapor pressure, or by chemical signals coming from root system (Chaves et al., 2009; Medrano et al., 2002).

Ramos (2009) observed that metabolism of orange plants were strongly affected by thermal regime, resulting in physiological changes related to photosynthesis, photoassimilates exportation, and photosynthetic pigments, which changed both fruit development and composition. However, during this experiment it was observed air temperatures below 10°C in the winter and higher than 38°C in the summer, which could cause long term modifications on plant physiology. Therefore, exposition of orange plants to temperatures higher than optimal can result in reduction of both photosynthesis rate and carbohydrate metabolism.

Reduction in CO<sub>2</sub> assimilation may be a consequence of closing the stomatal pores, and also because of possible photochemical damages in photosynthetic membranes (Guo et al., 2006). Increase of air temperature, and consequently rise of leaf temperature, promoted a reduction on photosynthetic rate because of increment on respiration rate (Lloyd and Farquhar, 2008). Results obtained from this work corroborated with Vilela (2012), which found substantial suppression on gas exchange in plants submitted to water stress. According to this author, water deficit resulted in proline accumulation in leaves, reduction on water potential, stomatal conductance, respiration rate, CO<sub>2</sub> assimilation, and mass accumulation. This reduction on photosynthetic activity caused by water stress occurred at same time of turgor pressure decrease (Larcher, 2000).

Following changes of trunk diameter and plant height, it was also observed reduction on fruit production. T-25 plants showed a reduction on both fruit size (-70%) and diameter (-112%) compared do Control (Table 2). Some authors concluded that reduction on productivity occurred when water stress was applied during both flowering and fruit set stages (Doorenbos and Kassam, 1994; Ginestar and Castel, 1996). In our experiment, water deficit was applied since vegetative growth stage, but reduction on fruit production was observed in plants grown under water deficit conditions.

It was found no changes on plant growth rates and production in plants irrigated with both 25% and 100% of crop evapotranspiration (Torrecillas et al., 1993; Domingo et al., 1996). However, those plants were irrigated with 100% of crop evapotranspiration after 'june drop' phase until stage of rapid fruit growth. It can be concluded that a controlled water deficit can improve water use efficiency.

Table 2. Number of leaves, plant height and trunk diameter of 'Navelate' orange plants evaluated monthly.

Treatments	Number of Leaves	Diameter (mm)	Height (cm)
Control	119	12.23	174
T-50	123	9.74	168
T-25	127	8.62	163

Water extracted through the root system is transported by the xylem and distributed across different plant parts before reaching the atmosphere through transpiration process that occurs in response to the energy gradient provided by solar radiation (FERNANDEZ et al.,

2011). In fact, plant growth and development are influenced by environmental factors which, among them, water availability plays a vital role as it intervenes in most physiological and biological processes.

Orange plants present morphological characteristics and physiological mechanisms which give considerably tolerance to water stress when compared to other perennial plants (Coelho et al., 2006; Pereira et al., 2009), but productivity are closely related to water availability. Since the photosynthetic rate are affected by water deficit, a reduction on both carbohydrate levels and fruit weight is expected (Cerqueira et al., 2004; Garcia-Sánchez et al., 2007), which can affect fruit quality.

The organic matter accumulated in a plant came from photosynthesis, it is represented 95% of its dry matter (Machado et al., 2005), and around 60% are in root system in orange seedlings (Santos et al., 2011). In this experiment, the highest growth rate (height and trunk diameter) occurred in plants with appropriate water supply (Control), which had higher photosynthetic rate, when compared to plants grown under water stress (T-50 and T-25). With water restriction, both T-50 and T-25 plants showed reduction on trunk growth (trunk diameter and height). These responses to acclimation limited water consumption by different tissues, helping to keep water status of those plants (Chaves et al., 2009).

The global climate change can be resulted in adverse conditions to plant growth and adapted to environmental stressed. Moreover, under such conditions they should acclimate and adapt to survive. The resistance to water deficit in citrus plants, for example, is based on tolerance (osmotic adjustment) and prevention (stomatal control) to water stress (Savé et al., 1995).

#### **4 CONCLUSIONS**

Orange plants submitted to 25% of irrigation of total field capacity showed reduction on stomatal conductance, transpiration and photosynthesis rate, which reduced plant growth (trunk diameter and plant height), both fruit production and quality. Best growth and production occurred in plants with any irrigation deficit. Deficient irrigation showed no changes in water use efficiency (normal and intrinsic), which means a good parameter of adaptation to stress condition. Water deficit, in any level, reduced both plant growth and fruit production.



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